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SKIN AND LUNG ABSORPTION OF TNT RESIDUES FROM COMPOSTED TNT-CONTAMINATED SOIL

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ABSTRACT

TNT residues in finished composts of TNT-contaminated soils cannot be extracted with aqueous or organic solvents. Based on this, composting is being used to remediate TNT-contaminated soils. The health hazards associated with dusts generated from such materials are not predictable since the stability of the TNT/compost bonds within biological systems is unknown.

To assess the bioavailability of composted TNT in the lungs, single doses of ^{14}C-TNT, soil spiked with ^{14}C-TNT, or dusts from compost generated with ^{14}C-TNT-spiked soils were administered to rats by intratracheal instillation. The appearance of ^{14}C in urine was taken as an indication of the release of TNT, or TNT residues, from compost particles. In rats instilled with ^{14}C-TNT, about 35% of the ^{14}C dose appeared in urine within 3 days. The ^{14}C level in urine decreased rapidly thereafter, and was undetectable by 4 weeks after treatment. Similar results were obtained with soil-treated rats. In contrast, after treatment with ^{14}C-labeled compost, only 2.3% of the total dose appeared in urine during the first 3 days. The ^{14}C from compost-treated rats continued to be excreted in urine for more than 6 months and the total amount in urine was comparable to that in TNT-treated animals. Determination of the radiolabel in tissues showed that ^{14}C accumulated in the kidney of rats treated with labeled compost but not in rats treated with ^{14}C-TNT or ^{14}C-TNT spiked soil. These results indicate that TNT residues in compost are not stable in the lungs.

To examine risks associated with skin contact, single doses of ^{14}C-TNT-labeled compost, ^{14}C-TNT-labeled soil, or ^{14}C-TNT were applied to excised pig skin mounted in *in vitro* evaporation/penetration cells. When pure ^{14}C-TNT in acetone solution was applied to excised pig skin, approximately 25% of the label penetrated the dermis within 48 hours. The corresponding values for soil and compost were 4% and 0.2%, respectively. Thus, the composting process reduced the skin penetration of TNT residues in soil.

INTRODUCTION

Laboratory scale experiments[1,2] and field tests[3] have shown that 2,4,6-trinitrotoluene binds to organic matter in compost. The characteristics of the "bond" have not been identified but the aromatic ring structure of TNT appears to remain intact during composting. While vulnerable to alkaline hydrolysis, the bulk of TNT residues in composts of TNT-contaminated soils appear to be particle bound as most of the TNT cannot be extracted with aqueous solutions or organic solvents.[3] Based on this, composting is being used at Umatilla Army Depot (UMDA) as a means for remediating TNT-contaminated soils. Exposure by skin contact or inhalation of dusts is possible during the preparation, processing and disposal of composted materials. However, virtually nothing is known about the effects of exposure to composts of TNT-contaminated soils.

TNT is a skin and respiratory tract irritant and can cause liver damage, methemoglobinemia, and other blood disorders. Long-term exposure caused tumors in female rats, but not in male rats or mice.[4,5] While the toxicity of TNT has been well studied, the health hazards associated with composted TNT cannot be predicted without experimental data since it is not known whether the TNT/compost "bonds" are stable in biological systems. Furthermore, should the compost/TNT bonds be unstable in the body, the toxicity could differ from that of TNT depending on whether TNT or a chemically altered form of TNT is released from the particulate matrix.

The studies described herein were designed to examine if TNT/particulate "bonds" in composts of TNT-contaminated soils remain stable in biological systems (i.e., are TNT, or TNT moieties, released from the compost matrix and freed to interact with biological tissues). Free TNT readily penetrates skin[6] and is absorbed rapidly through the lungs.[7] We used an *in vitro*/*in vivo* method for measuring skin penetration and an *in vivo* method to test lung absorption to examine bioavailability. The dermal procedure determined whether the TNT moiety from composts of TNT-contaminated soils in contact with artificial sweat can penetrate skin.

The lung absorption approach examined the bioavailability of composted TNT introduced into the lung by intratracheal instillation. The underlying premise of the lung study was that free TNT will be excreted in the urine and feces while TNT that is bound to particles will be excreted only in the feces. If the compost/TNT bonds are stable in the milieu of the respiratory tract, then the TNT will remain associated with compost particles, some of which will be cleared from the lung into the oral cavity. Most of the cleared material will be ingested to later appear in the feces. The remainder of the compost particles will remain in the lungs for an extended period. Thus, if the compost-bound TNT is not bioavailable, radiolabel should appear only in the lungs and feces.

If the TNT/compost bonds are broken in the lung, then the disposition of TNT (or TNT moieties) should be similar to that of neat TNT. It is known from the work of El-hawari et al.[7] that neat TNT is readily absorbed through the lung and appears rapidly in the urine and multiple

tissues. Thus, if the compost-bound TNT is bioavailable, radioactivity should appear in the urine as well as the feces and may be distributed to tissues throughout the body.

METHODS

Chemicals and test samples of soil and compost: Uniformly ring-labeled ^{14}C-TNT (98% pure) was obtained from NEN/DuPont, Boston, MA. Soil from an uncontaminated site at UMDA was provided by Roy Weston, Inc. (West Chester, PA). ^{14}C-TNT labeled soil was prepared freshly for each experiment by adding ^{14}C-TNT in tetrahydrofuran to uncontaminated soil and air-drying. For the preparation of compost, 20 mCi ^{14}C-TNT in 1 ml tetrahydrofuran was added to 2 g soil and air dried. The ^{14}C-TNT labeled soil was mixed with amendments and the compost generated onsite at UMDA by Roy F. Weston, Inc. The compost mixture contained 19.8% soil, 40.7% sawdust/alfalfa hay mixture (50:50), 3.9% chicken manure, and 24.2% cow manure. This was placed inside a small burlap bag in the middle of a non-radioactive aerated static pile of the same composition. After 90 days, the ^{14}C-TNT-labeled compost was removed from the burlap bag, and allowed to air-dry for 1 week in the dark. The dried compost was coarse ground in a Braun Model 201A coffee grinder for 5 minutes. The specific activity of the dried compost, 1.6 μCi/mg, was determined by oxidation in a Packard Model 306 Oxidizer. Analyses indicated that binding of the TNT to compost had occurred. Only about 1% of the radiolabel could be extracted into acetonitrile with sonication and about 21% could be removed by 2 hours of refluxing with 6 N HCl. The compost was stored in the dark at 4°C until needed.

Animals: Male F344 VAF/Plus rats, weighing between 180-200 g, were obtained from Charles River, Inc. Rats were housed in Bio-Clean cage enclosures and quarantined for one week. Specific pathogen free female Yorkshire pigs, weighing 15 to 20 kg were used for the skin penetration studies. Animals were allowed free access to feed and water throughout the study.

Lung study: Rats were anesthetized with Halothane in a carrier gas mixture of (O_2 and N_2O). Anesthetized rats were exposed by intratracheal instillation to single doses of neat ^{14}C-TNT (5 μg), freshly prepared ^{14}C-TNT labeled soil (5 mg), or composted ^{14}C-TNT labeled soil (5 mg). Each dose contained approximately 8 μCi ^{14}C and was delivered in 0.3 ml phosphate-buffered saline (PBS) containing 0.1% rat albumin (Sigma Chemical Corp.,St Louis, MO). The compost was homogenized (Tenbroek homogenizer) in PBS before administration to rats. Samples of the compost and soil doses were combusted in a Packard Model 306 Oxidizer and counted in a Beckman Model LS 5801 Liquid Scintillation counter to determine the precise quantity of ^{14}C administered to the rats.

Animals were individually housed in Nalgene^{R} metabolism cages for the collection of urine and feces. Cages were cleaned three times weekly. Urine was collected at 4, 24, 48, and 72 hours after exposure. For long-term TNT studies, urine was collected daily except on weekends when 3-day samples were

collected on Mondays. In the few instances when urine could not be collected during the 6-month compost study, ^{14}C levels were estimated by extrapolating between the nearest data points on either side of the data gap. For collection of tissues, animals were euthanized by i.p. injection of sodium pentobarbital (50 to 80 mg/kg) and exsanguination. Tissues (spleen, heart, liver, kidney, and lung) were excised, rinsed and dried; feces were dried and ground. Total radioactivity in feces and tissues was determined by oxidation and liquid scintillation counting.

Skin study: The in vitro skin penetration studies were conducted with the penetration/evaporation cell[8] shown in Figure 1. Two inch square, 500 μm thick sections of shaven pig skin were draped over the top of the penetration cell, epidermal surface up, and held in place with an O-ring. A peristaltic

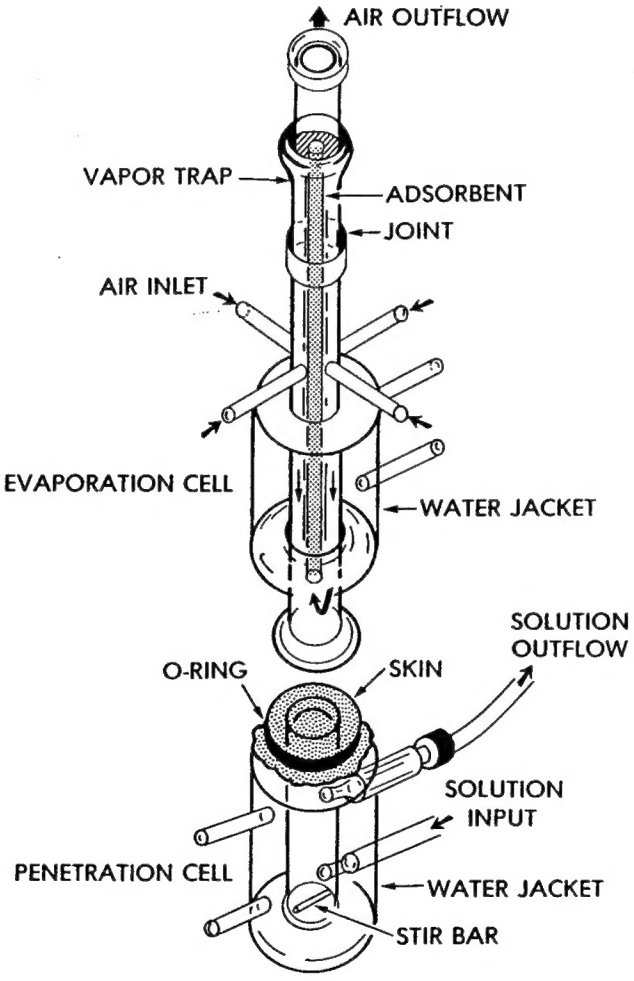

Figure 1. Flow-through skin diffusion apparatus used to measure ^{14}C-TNT penetration through excised pig skin. Culture medium is pumped into the receptor compartment on the dermal side of excised skin, mixed in the penetration chamber with a stir bar, and directed from the chamber to a fraction collector. The temperature is maintained by circulation of 37°C water through a jacket surrounding the penetration cell.

pump was used to perfuse the penetration cell with freshly prepared tissue culture medium. The medium kept the skin alive and also acted as a sink to collect the test material that penetrated the skin.

An evaporation cell, fitted with a vapor trap containing an adsorbent (Tenax GC), was clamped on top of the O-ring, leaving an exposed skin area of 0.8 cm^2. Air flow through the vapor trap was maintained at 600 ml/min with a vacuum pump. Radiometric assay of the adsorbent provided a measure of evaporative loss of test article from the skin surface.

Five μl of artificial sweat[9] was spread over the exposed epidermal surface followed by application of ^{14}C-labeled-soil or compost; the particles adhered to the dampened skin surface. To simulate a twelve hour exposure, soil or compost particles were removed from the skin surface by wiping with a dry cotton ball twelve hours after application. Forty eight hours after application, skin was removed from the penetration cells and the epidermis separated from the dermis. Skin sections were solubilized with hyamine solution (tissue solubilizer, Packard Instruments) heated to 50-60°C for approximately 1 hour and ^{14}C determined with a scintillation counter (Packard model CA 1900).

Six evaporation/penetration cells (A-F) were assembled on the day that a pig skin sample was obtained. The procedure was repeated on five separate days with skin from a different pig to give 6 replicates for each soil or compost exposure and 11 replicates for exposure to TNT. Cell A received a low soil dose (mean of 5 replicates: 1.7 mg/cm^2) cell B received a high soil dose (mean of 6 replicates: 5.3 mg/cm^2), cell C received low dose compost (mean of 6 replicates: 1.3 mg/cm^2) cell D received high dose compost (mean of 6 replicates: 5.4 mg/cm^2) and cells E and F received TNT (10 μg ^{14}C-TNT in 6.25 μl acetone/cm^2).

Statistical analysis. Excretion and tissue disposition in the rat were compared with the Student *t* test. Percutaneous penetration of soil and compost were compared using one-way ANOVA and Tukey's test. All determinations were carried out at the 0.05 significance level.

RESULTS

Lung study: Excreta

Marked differences were seen in the rates of ^{14}C excretion between rats intratracheally instilled with ^{14}C-labeled compost and those treated with ^{14}C-TNT or ^{14}C-labeled soil. Figure 2 shows the patterns of ^{14}C excretion by rats from each of the treatment groups during the first 3 days after exposure. ^{14}C levels were highest in urine at 4 hours for TNT-treated rats and at 24 hours for soil-treated rats. For both these treatment groups, ^{14}C reached a maximum in feces at 24 hours. Rats treated with compost excreted much less ^{14}C into both feces and urine than did rats treated with soil or TNT. A small peak in fecal radioactivity occurred at 2 days after compost treatment while, in urine, there was a steady excretion of low levels of ^{14}C.

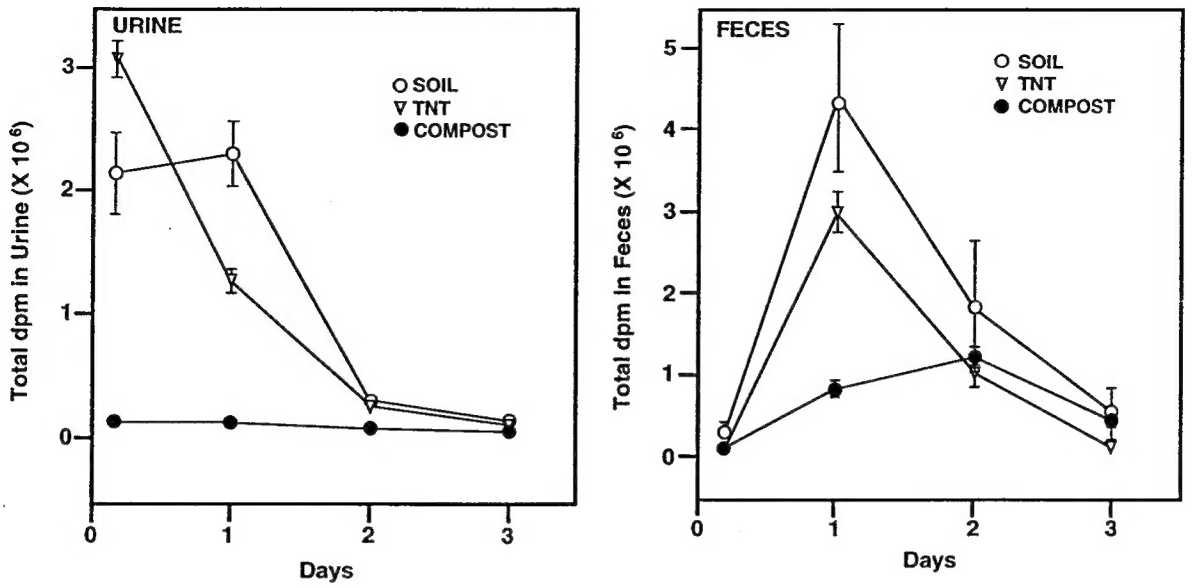

Figure 2. Excretion of ^{14}C by rats treated with ^{14}C-labeled TNT, soil and compost during the first 3 days after exposure. Urine and feces were collected at 4, 24, 48, and 72 hours. Dose = soil - 10.0 µCi; TNT - 8.0 µCi; compost - 8.0 µCi. Values are the mean +/- SD.

The total quantity of ^{14}C excreted in urine or feces during the first 3 days after exposure is given in Table 1. Animals treated with compost excreted about 15 times less ^{14}C into urine, and about 3 times less ^{14}C into feces, than did animals treated with ^{14}C-TNT. Soil-treated animals excreted about 1.5 times less ^{14}C than did TNT-treated rats during the first 3 days after treatment.

TABLE 1
Radiolabel Excreted During First 3 Days After Intratracheal Instillation

	Percent Administered Dose		
SAMPLE	FECES	URINE	TOTAL
COMPOST	15.0	2.3	17.3
TNT	46.7	35.0	81.7
SOIL	31.9	22.0	53.9

Relatively little ^{14}C was excreted by soil or TNT-treated rats after the third day following exposure (Table 2). With ^{14}C-TNT-treated rats, 35% of the ^{14}C dose appeared in urine within 3 days. The ^{14}C level in urine decreased rapidly thereafter, and was undetectable by 24 days after treatment. About 38% of the administered dose was recovered in the urine over the 24 day period. A similar ^{14}C excretion pattern occurred when rats were treated with ^{14}C-labeled soil, except that it took slightly longer (28 days) for the ^{14}C to completely disappear from the urine. (Total urinary excretion data for soil-treated rats are not shown because equipment constraints precluded obtaining measurements of urinary ^{14}C content at most time points during the 2nd and 3rd weeks after treatment).

In contrast to TNT- or soil-treated rats, only 2.3% of the ^{14}C in the administered compost dose appeared in urine during the first 3 days after treatment. However, low levels of ^{14}C continued to appear in the urine of compost-treated animals for more than 6 months and the total amount excreted in urine during the 6 months following treatment was comparable to that excreted by TNT-treated animals in 24 days (Table 2).

TABLE 2
Total Radiolabel Excreted In Urine After Intratracheal Instillation

	PERCENT ADMINISTERED DOSE			
SAMPLE	day 1-3	day 4-28	day 28-180	TOTAL
COMPOST	2.3	13.0	20.0	35.3
TNT	35.0	2.7	ND	37.7

ND = Not detectable

Lung study: tissues

The disposition of TNT residues in tissues was compared for TNT-, soil-, and compost-treated animals. ^{14}C levels were determined by oxidizing whole organs from rats sacrificed during the first 3 days and periodically thereafter. (Tissues from soil-treated rats were examined during the first 3 days after treatment only.) The ^{14}C levels in tissues were expressed as the percent of the total administered dose.

Figure 3 shows the distribution pattern of ^{14}C from the lungs. 99.6% of the administered dose disappeared from the lung within the first 4 hours after instillation of ^{14}C-TNT. By day 28, only 0.02% of the administered dose

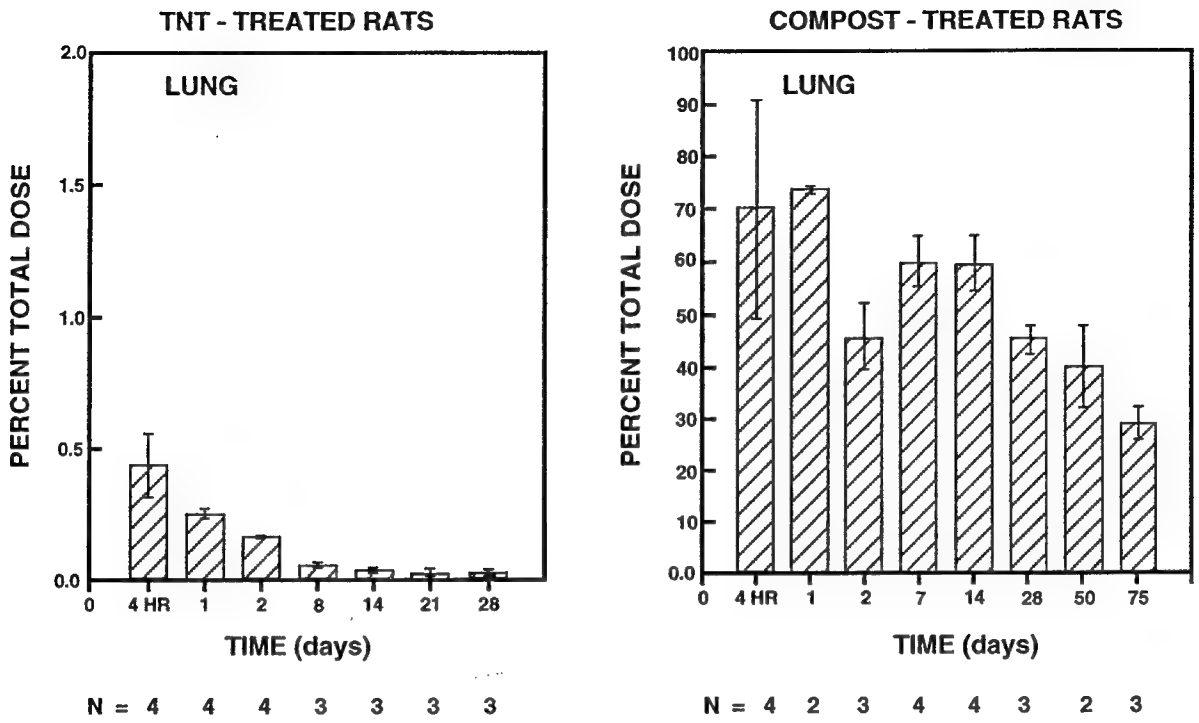

Figure 3. ^{14}C levels in lung tissue at various times after instillation of ^{14}C-labeled TNT or compost. Note that different scales are used for the vertical axis for compost- and TNT-treated rats. Dose = TNT - 8.3 μCi; compost - 6.61 μCi. Values are the mean +/- SD.

was detectable in the lungs. ^{14}C cleared slightly more slowly from the lungs of soil-treated than from TNT-treated rats (data not shown). Within the first 4 hours after treatment, 98.5% was cleared from the lung. This increased steadily until at 72 hours only 0.5% of the administered ^{14}C remained in the lung. The difference in the clearance rates between soil- and TNT-treated rats was not significant.

With compost, the continuous excretion of low levels of ^{14}C in urine or feces was paralleled by the slow clearance rate of ^{14}C from the lungs. At 24 hours, about 70% of the radiolabel was retained by the lung. There was a gradual decline in the ^{14}C level and, at day 75, 30% of the administered dose was still present.

The disposition of ^{14}C in liver, kidney, heart and spleen is shown in Figure 4. In TNT-treated rats, ^{14}C levels were highest in all organs tested at 4 hours after treatment. ^{14}C levels decreased rapidly thereafter. No differences were seen between the ^{14}C disposition in tissues of TNT- and soil-treated rats. In compost-treated rats, ^{14}C reached a higher level in tissues and remained elevated for a much longer period of time. In heart, spleen and

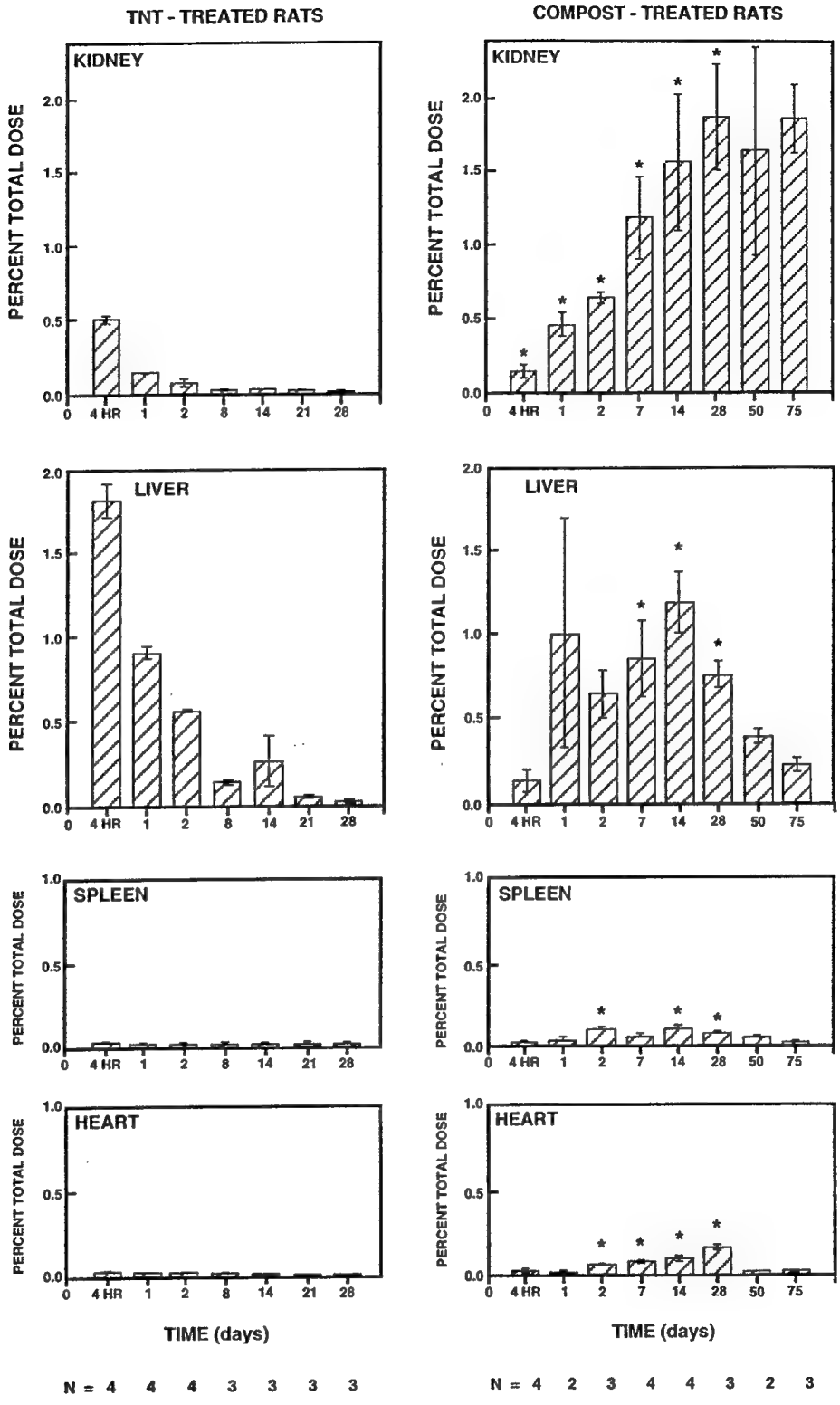

Figure 4. ^{14}C levels in tissues at various times after instillation of neat ^{14}C-TNT or compost prepared from soils labeled with ^{14}C-TNT.
** signifies significant difference ($p<0.05$) between ^{14}C levels in tissue taken from TNT- and compost-treated rats at the same time period after exposure.*
Dose = TNT - 8.3 µCi; compost - 6.61 µCi. Means +/- SE

liver, ^{14}C levels increased steadily and reached a peak at day 14 or 28 after administration, and declined thereafter. At day 28, kidney ^{14}C levels reached a plateau representing 2% of the administered dose. ^{14}C remained at this level until after day 75.

Skin penetration study

Figure 5 shows the amount of ^{14}C that penetrated *in vitro* skin slices over a 48 hour period following the application of ^{14}C-TNT in acetone (0.6 μCi) and the low doses of soil (1.8 μCi) and compost (1.6 μCi). For the acetone solution, 24% of the applied dose penetrated the skin, while the corresponding values for soil and compost were 4% and 0.2%, respectively. Values for soil and compost were significantly different ($p<0.05$). There was no significant difference between the percent penetration of ^{14}C from the high and low doses of soil or between the high and low doses of compost.

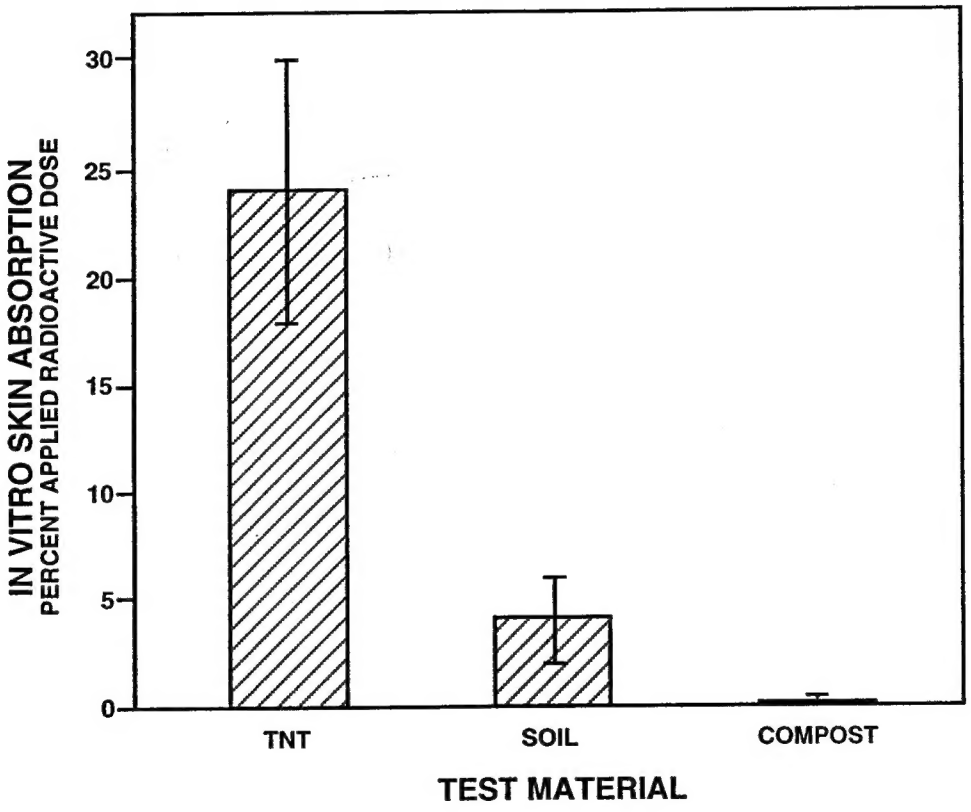

*Figure 5. In vitro percutaneous penetration of radioactivity from ^{14}C-labeled TNT, soil and compost. Percutaneous absorption is the sum of ^{14}C recovered in the dermis and the receptor fluid over a 48 hour period following the application of ^{14}C-TNT in acetone, and the low doses of soil and compost. Values are mean +/- SD. * The value for compost is significantly different from the value for ^{14}C-TNT labeled soil.*

In vitro results were verified by studies of the percutaneous absorption of ^{14}C-TNT following topical administration of soil or compost to the live pig (data not shown). There was an order of magnitude difference between the amount of ^{14}C excreted in urine from compost- and soil-treated pigs.

Evaporation was the major mode of loss of TNT applied to excised skin in acetone solution; 44% of the applied radiolabel evaporated during the 48 hour period after application (Figure 6). Less radiolabel was lost by evaporation when the carrier was soil; for the low soil dose, 17% of the applied radiolabel was lost by evaporation, while the value for percent evaporation was lower (5 +/- 3%) for the high soil dose. The amount of ^{14}C lost from compost by evaporation was insignificant.

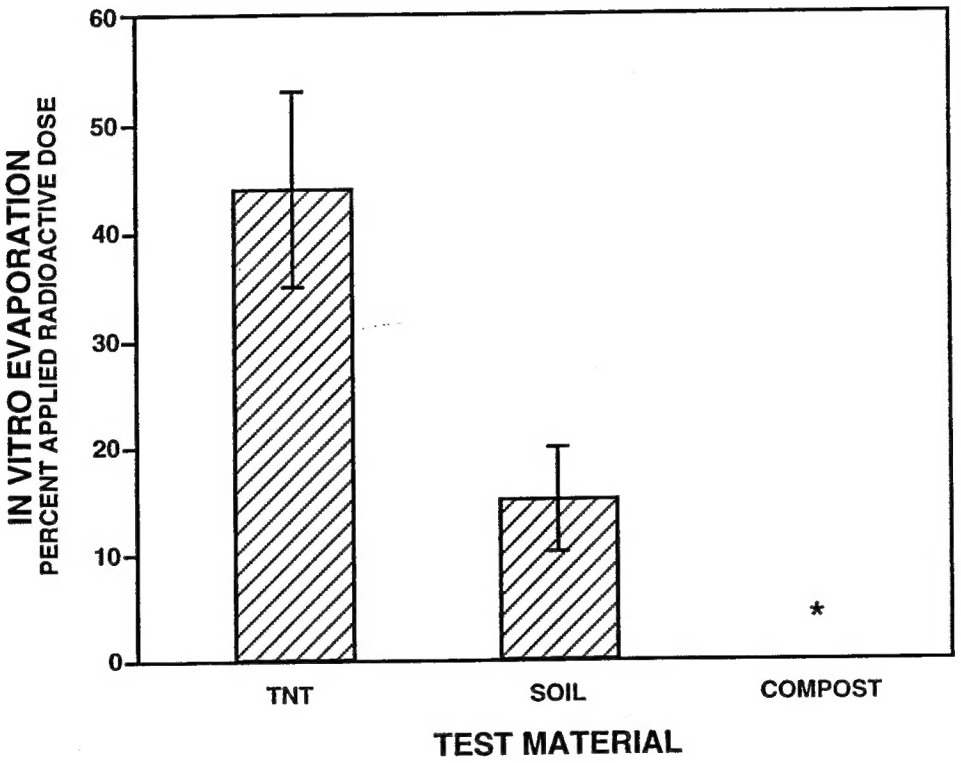

*Figure 6. Evaporation of ^{14}C from in vitro application of ^{14}C-labeled TNT, soil and compost. The percent evaporation was the percent of the applied ^{14}C dose recovered from the tenax GC adsorbent in the vapor traps. Mean values were significantly different ($p<0.05$). * value for compost = 0.02%.*

CONCLUSIONS

The basic premise of this work was that if TNT/compost bonds remain intact in the lungs, then the radioactivity that was not retained in the lung should be excreted in the feces but not in the urine. The finding that the

same amount of ^{14}C is excreted in urine from TNT- and compost-treated rats indicates that the TNT/compost bonds are broken down in the lungs. The release of TNT from the compost matrix is very slow, as evidenced by the marked differences in the retention of ^{14}C by lung tissue and the rates of urinary ^{14}C excretion between TNT- and compost-treated rats.

Tissues were exposed to higher levels of TNT (or TNT moieties) for a longer period of time in compost-treated rats than in rats exposed to ^{14}C-TNT. The differences between tissue disposition in compost-treated and TNT-treated animals were most striking for the kidney. Whereas the ^{14}C level in the kidney was 0.5% of the administered dose at 4 hours in TNT-treated rats and declined rapidly to 0.03% by day 3, the kidney contained between 1.5 and 2% of the administered dose for a period of more than 10 weeks in compost-treated rats. In on-going studies, we are examining the TNT residues present in kidney and urine to determine whether the accumulation can be attributed to differences in the proportion or in the chemical composition of TNT metabolites in TNT and compost-treated rats.

In terms of risk, these experiments show that, while precautions should be taken against inhaling dusts from composts of TNT-contaminated soils, there may be much less risk associated with skin exposure. In vitro skin penetration studies designed to simulate 12 hour exposures, demonstrated that the dermal penetration of ^{14}C was markedly lower when skin was treated with ^{14}C-labeled compost than with ^{14}C-labeled soil (0.2% vs 4.0%). These results were confirmed by in vivo determinations of ^{14}C skin peneetration and excretion in the pig.

In conclusion, TNT moieties in composts of TNT-contaminated soil are bioavailable in the lungs. It is not known whether the bonds between TNT and compost particles breakdown by active (e.g., enzymatic) or passive mechanisms (e.g., exchange reactions) in the lungs or whether the TNT moiety that is released from compost is more or less toxic than TNT itself. Substantial levels of the TNT moiety can accumulate in kidneys for an extended period of time and further study is essential to identify the TNT moieties released from the compost in order to assess the potential health risks associated with exposure to composts of TNT-contaminated soils.

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